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Docket Number: 1011/11

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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TITLE OF THE INVENTION (280 characters max)

SCREENING FOR INNOVATIVE TREATMENT FOR POST-TRAUMATIC STRESS DISORDER USING MULTI DIMENSIONAL ANIMAL MODELS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, DC 20231

P19LARGE/REV04

SCREENING FOR INNOVATIVE TREATMENT FOR POST-TRAUMATIC STRESS DISORDER USING MULTIDIMENSIONAL ANIMAL MODELS

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FIELD OF INVENTION

Post-traumatic stress disorder (PTSD) is a common condition, which affects about 6% of the general population and has severe impact on the quality of life. (Kessler et al., 1995). PTSD is currently defined by the coexistence of three clusters of symptoms: re-experiencing, avoidance and hyper-arousal, which persist for at least one month, in survivors of a traumatic event (American Psychiatric Association, 1994).

To date, there is still an ongoing search for treatment of PTSD. Although sertraline, the selective serotonin re-uptake inhibitor approved for PTSD, was found to be clinically effective, the result is still far from providing a full remission (Brady et al., 1995). Abnormal activity of the autonomic nervous system (ANS) (Stein et al., 2000) and of the hypothalamic-pituitary-adrenal (HPA) axis (Yehuda et al. 1991) has been suggested as the basis of some of the characteristic features of PTSD. However, the findings concerning adrenocortical dysfunction in PTSD patients remain unclear. Pitman and Orr (1990) reported high 24-hour urinary cortisol in veterans with PTSD compared to normal controls with combat experience. Conversely, four studies reported lower 24-hour urinary cortisol in PTSD patients compared to normal controls, and to depressed patients (Yehuda et al. 1990). Young et al. (1995) reported enhanced pituitary proopiomelanocortin (POMC) messenger ribonucleic acid (mRNA) expression and corticotropin (ACTH) storage. Kosten et al. (1997) also reported elevated epinephrine level during PTSD patients as compared to major depressive disorder, paranoid schizophrenia and undifferentiated schizophrenia.

DESCRIPTION OF INVENTION

Current studies of the available animal models for PTSD ignore the well-accepted clinical findings that only a minority (about 20%) of the individuals that are exposed to a traumatic event will eventually develop PTSD (Yehuda et al., 1995). The novelty of this invention is the ability to separate and identify those

animals, which, after exposure to trauma, will develop symptoms mimicking post-traumatic stress response. Therefore, the objectives of the present method are:

1. To recognize and analyze specifically the behavior of the animals which respond in an overly exaggerated way after exposure to events that remind the traumatic event. This most effectively mimics post- traumatic stress response as expressed in humans.

2. To characterize the PTSD model by three separate clusters of symptoms as found in clinical PTSD:
   - Re-experiencing
   - Avoidance
   - Hyper-arousal

3. To test different drugs on these three different clusters of PTSD symptoms, and to analyze the response accordingly.

4. To test preventative methods (including pharmacological interventions) of PTSD with intervention prior, during, or after the exposure.

5. To elucidate biochemical alterations before and after drug treatment in relation to each of the three characterized clusters of PTSD symptoms.

6. To test combined drug treatments on the basis of data derived from 2,3 and 4.

DESCRIPTION OF METHOD

Animals:
Adult male Sprague-Dawley rats weighing 150-200 gr. are used. Animals are habituated to the housing conditions and testing area for at least ten days. During that time, rats are handled once daily; the handling consists of picking the rats up with a gloved hand. The animals are housed four per cage (two rats that are exposed to stress and two additional rats for social behavior sampling) in an animal room with stable temperature, reversed 12 hours light/dark cycle, with *ad libitum* food and water. All testing is performed during the dark phase using a dim light.

Initial exposure:
Rats are exposed to a blanket with the smell of a cat for 30 minutes in a restricted environment i.e. cage or bordered area; this time point is called "zero time".

Re-exposure:
The rats are again exposed to a blanket, with a similar texture but without the smell, one week after initial exposure and again after one month. The controls in our model are rats that will be re-exposed to the blanket but will not go on to develop PTSD as opposed to animals that will display PTSD symptoms.
It is important to note that this model can be extended to any animal species exposed to any natural occurring stress (exposure to a predator underwater stress etc.)

BEHAVIORAL MEASUREMENT
Before exposure to the stressor, the animal is tested (videotaped) in an open field chamber alone (5 min) with its habituated companion rat (5 min) and during hyperarousal event (5 min). Immediately following re-exposure (both at "zero time", after one week and after one month) the rats are re-tested in an open field chamber in same conditions and paradigm. The animals are then screened according to the following parameters:

1) Self behaviour:

Observed Behaviour	Normal Threshold (% of total time)	PTSD (% of total time)
Freezing	0.5-5	20-80
Grooming	7-13	4-13
Sniffing	6-14	11-22
Climbing over	12-17	2-4
Staying in corner	29-43	51-75

2) Social interaction and avoidance:
The re-exposed animal is paired with a rat which has not been exposed to trauma and the following social interactions are observed.

Observed Behaviour	Normal Threshold (% of total time)	PTSD (% of total time)
Freezing	25-50	60-75
Grooming	11-17	0-2
Sniffing	8-16	26-42
Climbing over	6-12	7-13
Crawling under	0-2	2-4
Genital Investigation	0-2	0-2
Staying in corner	23-37	25-45

3) Hyper-arousal:
The re-exposed animal is subjected to loud clap, bang or any loud noise.

Observed Behaviour	Normal Threshold (% of total time)	PTSD (% of total time)
Freezing	5-70	90-100

BIOCHEMICAL TESTS

ACTH and corticosterone are measured in plasma samples with RIA kits. Biochemical testing is performed one week after the last exposure. PTSD rats according to behavioral measures showed either increased (X7) or decreased (X0.2). The HPA axis is indeed afflicted by stress. In the literature there are findings which report high 24-hour urinary cortisol in veterans with PTSD (Morgan et al, 2000) along with studies that report lower 24-hour urinary cortisol in PTSD patients (Yehuda et al, 1992).

POTENTIAL OF MODEL FOR DRUG SCREENING

The method enables screening for potential drugs in the treatment of PTSD including SSRIs, nerve-protecting agents, medications which alter the HPA axis, beta-blockers, etc. These medications are given either immediately after original exposure or after first re-exposure with the intention of testing as to whether these compounds prevent or decrease intensity or frequency of PTSD symptoms. Testing is carried out as previously described. Criteria for improvement is a decrease by more than 25% in each of the systems tested.

CONCLUSION:

Using the criteria outlined in this model, only 12% of the rats that are exposed to stress and then re-exposed develop symptoms characteristic of PTSD. Measurement of ACTH and corticosterone in plasma samples of the rats a week after the last behavioral measurement (month point time) show inconsistencies. Both these findings are consistent with human PTSD, emphasizing the reliability and validity of the model and providing new parameters which may correlate with the disease.

REFERENCES

American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders, 4th edn (DSM-IV) (American Psychiatric Press: Washington, DC, 1994).

Brady KT, Sonne SC, Roberts JM, Sertraline treatment of comorbid posttraumatic stress disorder and alcohol dependence. J Clin Psychiatry (1995) 56:502-5.

File SE, the interplay of learning and anxiety in the elevated plus-maze, Behav Brain Res (1993) 20:58(1-2):199-202.

Kessler RC, Sonnega A, Bromet EJ et al, Posttraumatic stress disorder in the National Comorbidity Survey, Arch Gen Psychiatry (1995) 52:1048-60.

Kosten TR, Ziedonis DM, Substance abuse and schizophrenia: editors' introduction, Schiz Bull (1997):23(2):181-6.

Morgan CA, 3rd, Wang S, Mason J, et al (2000): Hormone profiles in humans experiencing military survival training. *Biol Psychiatry* 47:891-901.

Morris RGM., development of a water-maze procedure for studying spatial learning in the rat, Journal of neuroscience methods 11,47-60.

Pitman RK, Orr SP, Twenty-four hour urinary cortisol and catecholamin excretion in combat-related posttraumatic stress disorder, Biol Psychiatry (1990) 15:27(2)245-7.

Stein DJ, Seedat S, Van der Linder, Kaminer D, Pharmacotherapy of post-traumatic stress disorder In "Post-traumatic Stress Disorder" Ed Natt D, Davidson J, Zohar J, Martin Dunitz Ltd.2000 London.2000 pp131-46.

Yehuda R, Giller EL, Southwick SM, Lowy MT, Mason JW, Hypothalamic pituitary-adrenal dysfunction in posttraumatic-stress-disorder, Biol Psychiatry (1991) 30:(10) 1031-48.

Yehuda R, McFarlane AC, Conflict between current knowledge about posttraumatic stress disorder and its original conceptual basis, Am J Psychiatry (1995) 152:1705-13.

Yehuda R, Southwick S, Giller EL, Ma X, Mason JW (1992): Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. *J Nerv Ment Dis* 180:321-5.

Yehuda R, Southwick SM, Nussbaum G, Wahby V, Giller EL Jr, Mason JW, Low urinary cortisol excretion in patients with posttraumatic stress disorder. J Nerv Ment Dis (1990) 178(6):366-9.

Young EA, Kwak SP, Kottak J, Negative feedback regulation following administration of chronic exogenous corticosterone, J Neuroendocrinol (1995) 7(1):37-45.

WHAT IS CLAIMED IS:

1. A method for producing an animal with symptoms mimicking post-traumatic stress disorder, the method comprising:

a. exposing the animal to a trauma-inducing stimulus

b. re-exposing the animal to the stimulus

c. determining if the animal exhibits at least one behavioral response characteristic of post traumatic stress disorder, and

d. diagnosing the animal as having post traumatic stress disorder if the animal exhibits at least one behavioral response characteristic of post traumatic stress disorder.